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Provisional Application Cover Sheet

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This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(b)(2).

Docket Number: Q3351		Type a plus sign (+) inside this box	+

Inventor(s)/Applicant(s)			
Last Name	First Name	Middle Initial	Residence (City and either State or Foreign Country)
Woo	Y	J	Lafayette Hills, PA
Title of the Invention (280 Characters Maximum)			
Ethyl Pyruvate Reduces Free Radical Production and Preserves Cardiac Function in a Rat Model of Off-Pump Coronary Bypass			
Correspondence Address			
University of Pennsylvania Center For Technology Transfer 3160 Chestnut Street Suite 200			
City: Philadelphia	State: Pennsylvania	Zip Code: 19104 - 6283	Country: US
Enclosed Application Parts (*check all that apply*)			
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[] Our Check No. ____ is enclosed to cover the Provisional filing fee. A duplicate copy of this sheet is enclosed. [X] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 13-2489. A duplicate copy of this sheet is enclosed. [] Payment by credit card. Form PTO-2028 is attached.	Provisional Filing Fee Amount ($)	$ 80.00	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[] No
[] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature: [signature] Date: 10/27/03
Typed or Printed Name: Y. Joseph Woo

[] Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

PROVISIONAL APPLICATION SUBMISSION TO USPTO - CONTENTS PAGE

Penn Docket Number	:	Q3351
First-named Inventor	:	Woo
Submission Date	:	October 27, 2003
Prepared by	:	Andrew Liken

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Total Number of Pages : 10

Q3351 / RBM

Ethyl Pyruvate Reduces Free Radical Production and Preserves Cardiac Function in a Rat Model Of Off-Pump Coronary Bypass

Matthew D. Taylor MSIII, Todd J. Grand BS, Jeffrey E. Cohen, Vivien Hsu, Suzanne Zentko MD, Mark F. Berry MD, Y. Joseph Woo, MD

University of Pennsylvania School of Medicine,

Dept. of Surgery

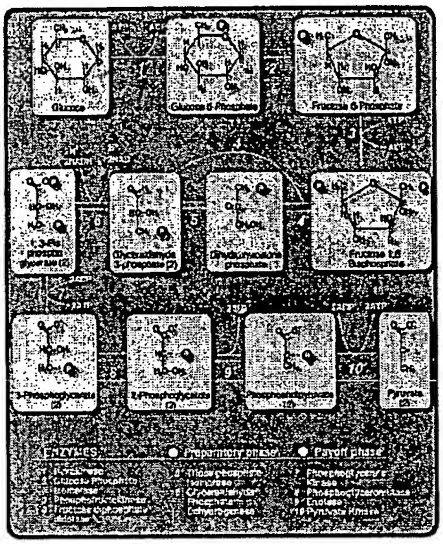
American Heart Association Scientific Sessions Nov. 2003

Background

Off-pump coronary artery bypass grafting is associated with transient periods of myocardial ischemia during revascularization. Such ischemia puts the myocardium at risk for contractile dysfunction and injury associated in part by the metabolic depletion of high energy phosphates. In.addition, reperfusion following revascularization is associated with myocardial oxidative injury mediated by reactive oxygen species.

Pyruvate, a key glycolytic intermediate, has been shown to be an effective scavenger of hydrogen peroxide and hydroxyl radical.[1,2] However, it's function as an antioxidant is limited by poor stability in aqueous solution.[3,4] Ethyl pyruvate, a common food additive, has enhanced lipophilic character and in contrast to pyruvate, is very stable in calcium containing aqueous solutions. Previous work utilizing a model of prolonged myocardial ischemia/reperfusion injury demonstrated that ethyl pyruvate enhanced myocardial ATP production, attenuated oxidative injury and infarct size, and preserved cardiac function.[5] The purpose of this study was to investigate the efficacy of ethyl pyruvate as a myocardial protective agent in a rat model of off-pump coronary artery bypass grafting .

Pyruvate- Glycolytic ATP Generator



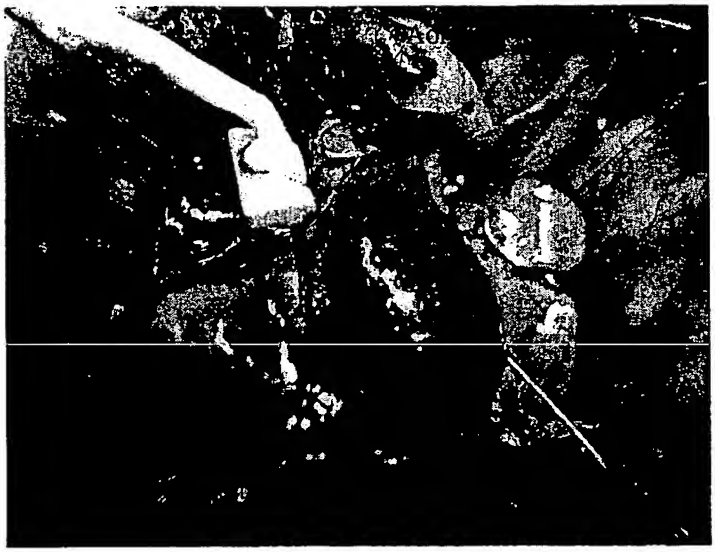
- Pyruvate supplementation produces increased NAD+
- NAD+ is the electron acceptor for reaction 6 producing 1,3-bisphosphoglycerate
- The enzyme, phosphoglycerate kinase, catalyzes reaction 7, in which 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate generating ATP

Source: Biocarta, www.biocarta.com

Methods

Male Wistar rats were anesthetized and mechanically ventilated with a respirator. The right femoral vein was isolated and an intravenous catheter was inserted for administration of treatment solutions. A median sternotomy was performed and the pericardium was reflected exposing the heart. A miniature pressure/volume conductance catheter was inserted into the left ventricular cavity via the apex for hemodynamic measurements. In addition, a flow probe was placed around the ascending aorta to measure cardiac output. (Figure 1) Animals were subjected to transient ischemia via a 10 minute occlusion of the left anterior descending coronary artery followed by 10 minutes of reperfusion. Animals received an IV bolus of Ringer's solution as a control (n=10) or Ringer's ethyl pyruvate (n=10) immediately before the initiation of both ischemia and reperfusion. Hemodynamic parameters were reported as a percentage of baseline measurements. Myocardial ATP levels were determined in the ischemic region following 10 minutes of ischemia (n=10). Myocardial lipid peroxidation , a measure of oxidative stress, was determined in the LV region subjected to ischemia as well as a region not subjected to ischemia as an internal control (n=10).

Figure 1- Photograph of experimental setup for the determination of cardiac function



Results

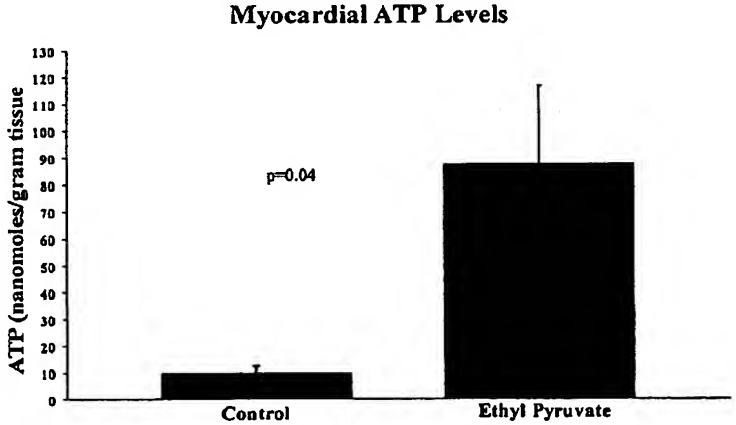


Figure 2- Myocardial ATP levels determined in the ischemic region following 10 minutes of ischemia (n=5, in both treatment group). Ethyl pyruvate treatment significantly increased ATP levels in ischemic myocardium compared to controls (87.6±29.2 vs. 10.0±2.4 nanomoles/g, *p=0.04).

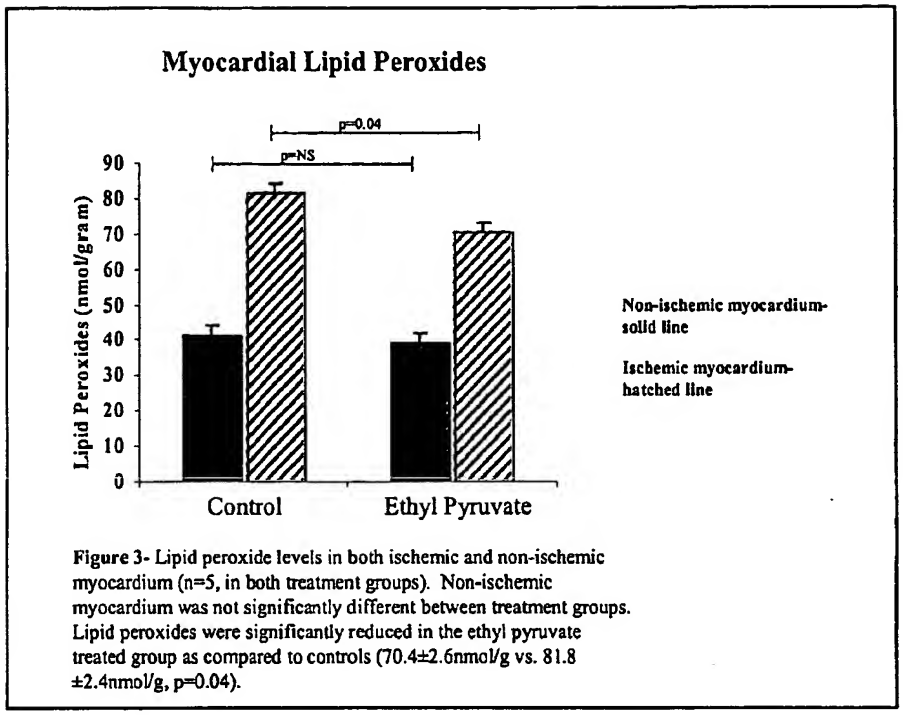


Figure 3- Lipid peroxide levels in both ischemic and non-ischemic myocardium (n=5, in both treatment groups). Non-ischemic myocardium was not significantly different between treatment groups. Lipid peroxides were significantly reduced in the ethyl pyruvate treated group as compared to controls (70.4±2.6nmol/g vs. 81.8 ±2.4nmol/g, p=0.04).

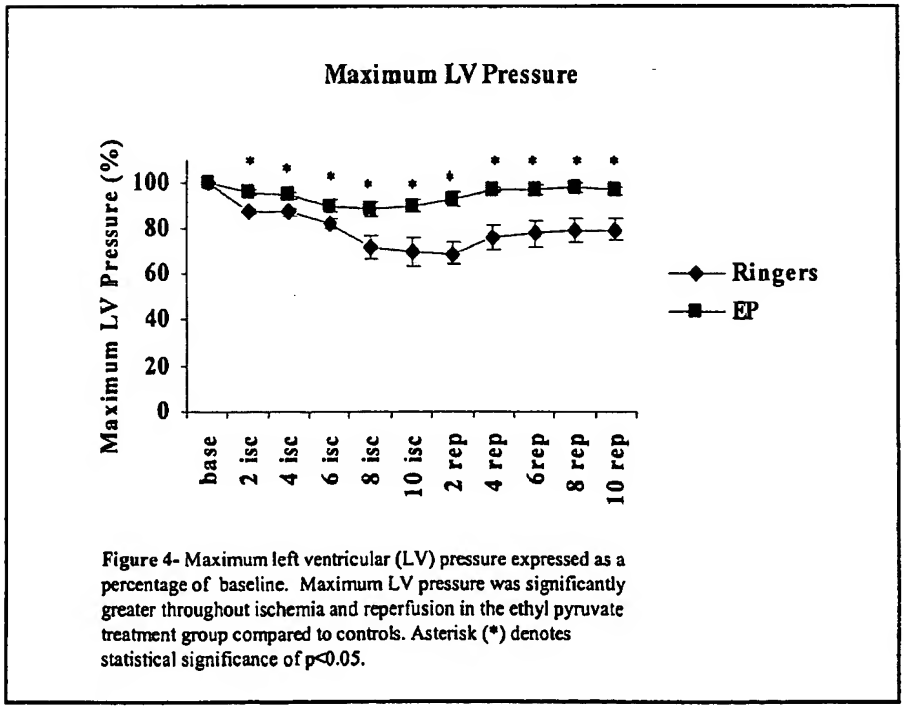


Figure 4- Maximum left ventricular (LV) pressure expressed as a percentage of baseline. Maximum LV pressure was significantly greater throughout ischemia and reperfusion in the ethyl pyruvate treatment group compared to controls. Asterisk (*) denotes statistical significance of $p<0.05$.

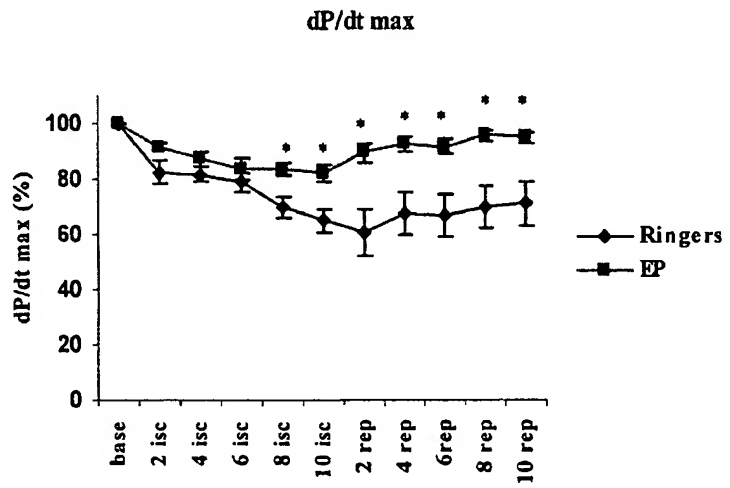


Figure 5- dP/dt max, a measure of contractility, expressed as a percentage of baseline. dP/dt max was significantly greater after 8 minutes of ischemia and throughout reperfusion in the ethyl pyruvate treatment group compared to controls. Asterisk (*) denotes statistical significance of $p<0.05$.

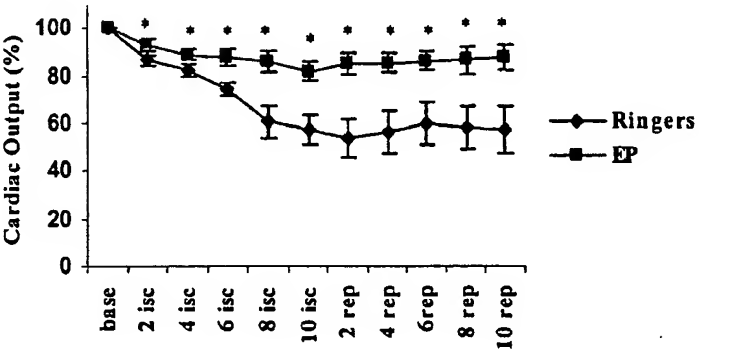


Figure 6- Cardiac output expressed as a percentage of baseline. Cardiac output was significantly greater throughout ischemia and reperfusion in the ethyl pyruvate treatment group compared to controls. Asterisk (*) denotes statistical significance of $p<0.05$.

Conclusion

- In a rat model of off-pump coronary artery bypass utilizing transient myocardial ischemia and reperfusion, ethyl pyruvate enhanced the myocardial energetic state by elevating ATP levels, reduced myocardial oxidative injury, and preserved myocardial function.

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Ethyl pyruvate preserves cardiac function and attenuates oxidative injury after prolonged myocardial ischemia.

Y. Joseph Woo, MD [1]
Matthew D. Taylor, BS [1]
Jeffrey E. Cohen [1]
Vasant Jayasankar, MD [1]
Lawrence T. Bish, BS [2]
Jeffrey Burdick BS[1]
Timothy J. Pirolli [2]
Mark F. Berry, MD [1]
Vivian Hsu[1]
Todd Grand, BS [1]

University of Pennsylvania School of Medicine
Departments of Surgery [1] and Physiology [2]

Corresponding Author:

Y. Joseph Woo, M.D.
Assistant Professor of Surgery
Director, Minimally Invasive and Robotic Cardiac Surgery Program
Division of Cardiothoracic Surgery
Department of Surgery
University of Pennsylvania
Silverstein 4
3400 Spruce St
Philadelphia PA 19104

215-662-2956
215-349-5798 FAX
wooy@uphs.upenn.edu

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Abstract

Objective: Myocardial injury and dysfunction following ischemia are mediated in part by reactive oxygen species. Pyruvate, a key glycolytic intermediary, is an effective free radical scavenger, but unfortunately, is limited by aqueous instability. The ester derivative, ethyl pyruvate, is stable in solution and should function as an antioxidant and energy precursor. This study sought to evaluate ethyl pyruvate as a myocardial protective agent in a rat model of ischemia/reperfusion injury.

Methods: Rats underwent 30minute ischemia and 30minute reperfusion of the left anterior descending coronary artery territory. Immediately prior to both ischemia and reperfusion, animals received an intravenous bolus of either ethyl pyruvate (n=26) or vehicle control(n=26). Myocardial high energy phosphate levels were determined by ATP assay, oxidative injury was measured by lipid peroxidation assay, infarct size was quantified by triphenyltetrazolium chloride staining and cardiac function was assessed in vivo.

Results: Ethyl pyruvate administration significantly increased myocardial ATP levels compared to control, 87.6±29.2nanomoles/g vs. 10.0±2.4nanomoles/g, p=0.03. In ischemic myocardium, ethyl pyruvate reduced oxidative injury compared to control: 63.8±3.3nmol/g vs. 89.5±3.0nmol/g, p<0.001. Ethyl pyruvate diminished infarct size as a percentage of area at risk: 25.3±1.5% vs. 33.6±2.1%, p=0.005. Ethyl pyruvate improved myocardial function compared to controls: Max Pressure 86.6±2.9mmHg vs. 73.5±2.5mmHg, p<0.001, Max dP/dt 3518±243mmHg/sec vs. 2703±175mmHg/sec, p=0.005, dV/dt 3097±479µl/sec vs. 2120±287µl/sec, p=0.04, Ejection Fraction 41.9±3.8% vs. 31.4±4.1%, p=0.03, Cardiac Output 26.7±0.9ml/min vs. 22.7±1.3ml/min,

p=0.01, and End systolic pressure volume relationship slope 1.08± vs. 0.59± , p=0.02.

Conclusions: In this study of myocardial ischemia reperfusion injury, ethyl pyruvate enhanced myocardial ATP levels, attenuated myocardial oxidative injury, decreased infarct size, and preserved cardiac function.

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